Review Article
http://dx.doi.org/10.5115/acb.2012.45.3.141
pISSN 2093-3665 eISSN 2093-3673
Anatomy & Cell Biology acb

Mechanism of experimental autoimmune encephalomyelitis in Lewis rats: recent insights from macrophages

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Abstract: Experimental autoimmune encephalomyelitis (EAE) in Lewis rats is an acute monophasic paralytic central nervous system disease, in which most rats spontaneously recover from paralysis. EAE in Lewis rats is induced by encephalitogenic antigens, including myelin basic protein. EAE is mediated by $CD4^+$ Th1 cells, which secrete pro-inflammatory mediators, and spontaneous recovery is mediated by regulatory T cells. Recently, it was established that classically activated macrophages (M1 phenotype) play an important role in the initiation of EAE, while alternatively activated macrophages (M2 phenotype) contribute to spontaneous recovery from rat EAE. This review will summarize the neuroimmunological aspects of active monophasic EAE, which manifests as neuroinflammation followed by neuroimmunomodulation and/or neuroprotection, with a focus on the role of alternatively activated macrophages.

Key words: Experimental autoimmune encephalomyelitis, Lewis rats, Macrophages, Neuroimmunomodulation, Regulatory T lymphocytes

Received May 22, 2012; Revised June 1, 2012; Accepted June 12, 2012

Introduction

Experimental autoimmune encephalomyelitis (EAE) has been studied for several decades as a model of autoimmune central nervous system disease, particularly human demyelinating multiple sclerosis (MS) [1-4]. The general features of EAE in animal models do not fulfill all characteristics of human MS although the neuropathologic features of EAE lesions occasionally display patterns similar to those of human MS [3, 4]. Thus, EAE is an alternative model for human MS because some of its features match those of human MS.

The advantages and disadvantages of animal models of EAE have been well reviewed and depend on pathology, T-cell phenotype, and the production of pro- and anti-inflammatory molecules [2, 3, 5, 6]. EAE may be induced in susceptible animals, including mice and rats, by immunization with brain tissue-specific antigens, including proteolipid protein (PLP), myelin basic protein (MBP), and myelin oligodendrocyte glycoprotein (MOG). Even though the autoimmune mechanism of EAE is similar in mice and rats, the pattern of EAE pathogenesis in mice [7] is slightly different from that in Lewis rats. Even among rat models, the pathology of Lewis rat EAE is distinct from that of Dark Agouti rats, which shows a recurrent pattern [8-10]. Thus, this review will limit the discussion to Lewis rat EAE.

After immunization of susceptible Lewis rats with brain

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tissue antigens plus complete Freund's adjuvant (CFA), the animals develop hind limb paralysis clinically [11]. At that time, neuropathological lesions are found mainly in the spinal cord and brain stem, but only rarely in the cerebrum. EAE lesions are characterized by edema, infiltration of mononuclear cells, and gliosis [12], but rarely demyelination. Animals afflicted with EAE experience spontaneous recovery from paralysis. Thereafter, the recovered animals are resistant to re-induction of EAE, showing signs of immunization. Instead of active induction of EAE upon immunization with myelin antigens, EAE can be passively induced by the transfer of encephalitogenic T cells; this is known as passive EAE [13-15].

Even though rat EAE does not show typical demyelination in target organs, the rat EAE model has generated considerable information regarding T-cell immunology and neurobiology, because in Lewis rats EAE is induced uniformly.

The present review will discuss the general features of active monophasic EAE in Lewis rats and highlights a novel view of the dual phenomenon of neuroinflammation and neuroprotection, with a particular emphasis on the macrophage phenotypes present during the course of EAE.

Encephalitogenic Antigens in Acute EAE in Lewis Rats

Acute monophasic EAE in Lewis rats is induced by immunization with whole homogenate of spinal cord tissue from guinea pigs with CFA [16], although rat MBP is also encephalitogenic [13, 17, 18]. Homogenate of guinea pig spinal cord tissue has been known to be more encephalitogenic than that of rats. Spinal cord homogenate contains fewer encephalitogenic antigens than does purified antigen, including MBP, PLP, and MOG. Thus, purified MBP, PLP, and MOG antigens, or synthetic peptides corresponding to encephalitogenic epitopes, have been used in studies of rat EAE. Of these, MBP is most commonly used as the immunogen for rat active EAE, and so will be the main antigen discussed in this review. The encephalitogenicity of myelin proteins and the general features of EAE in Lewis rats are well-reviewed elsewhere [13].

Neuropathogenesis of Active EAE in Lewis Rats

Behavioral changes

After active immunization with MBP plus CFA supplemented with *Mycobacterium tuberculosis*, immunized rats show tail atony by days 8-12 post-immunization (p.i.), hind limb paralysis–and in a few cases, forelimb paralysis–by days 13-15 p.i.; thereafter the rats show spontaneous recovery from paralysis [11, 12, 19]. Because of the appearance of typical behavioral paralysis in immunized rats within two weeks, this model has been used for pilot studies of anti-inflammatory drugs, including phenidone [20] and sodium salicylate [21]. Hyperacute severe EAE was induced by pertussis toxin in MBP-immunized Lewis rats [22].

Lesion distribution

Histopathological findings in EAE lesions are well matched with those of behavioral changes in EAE-afflicted rats. Perivascular cuffings were primarily found in the caudal lumbar spinal cord [23], but a few were found in the cerebrum, which may have been involved in the hind limb paralysis. Perivascular cuffings were occasionally found around ventricles, where extravasation easily occurs. Even though pathological changes, including perivascular cuffing, were not consistently found in the brain, it has been reported that cognitive deficits [24] were induced in EAE-afflicted animals and that certain signals, including cannabinoid receptors [25], were altered in EAE-afflicted brains, possibly influenced by inflammatory mediators secreted by inflammatory cells.

In Lewis rat EAE, demyelination is not prominent. Few, if any, demyelination events were found in the root entry zone [26]. Thus, the paralytic behavior seen in rat active EAE was induced by physical injury from infiltrating cells as well as by edema caused by blood-brain barrier disruption, rather than by demyelination in the spinal cord or brain. These findings are regarded as a limitation of animal models of human demyelinating MS [7].

Cell phenotypes in EAE lesions

In Lewis rats, MBP-induced EAE is characterized by infiltration of $CD4^+$ T cell receptor (TCR) alpha/$beta^+$ [12], TCR Vβ 8.2^+ T helper cells [27] and other cell types, including macrophages [3, 28, 29], B cells [3], natural killer cells, and mast cells [3]. Of these, $CD4^+$ TCR alpha/$beta^+$, TCR Vβ 8.2^+ T cells are one of the key cell types in EAE, because treatment

with antibodies against TCR alpha/beta [30] and TCR Vβ 8.2 [31] ameliorated EAE. With regard to the macrophages in rat EAE lesions, monocyte-derived macrophages in concert with T cells are thought to play a crucial role in the tissue damage seen in EAE because blocking of the type 3 complement receptor (CR3), or elimination of macrophages, suppressed rat EAE [32, 33]. Recently, B cells have been shown to partly contribute to EAE pathogenesis by presenting antigen and providing co-stimulation to T cells, producing cytokines and antibodies [3].

Cytokine profiles

In the past decades, research has focused on secretion of pro-inflammatory cytokines by the cells infiltrating EAE lesions at various stages [19, 34, 35]. Pro-inflammatory cytokines, including interferon-gamma [36], tumor necrosis factor (TNF)-alpha [37], interleukin (IL)-1-beta [38, 39], and IL-6 [40], are known to be associated with EAE induction, while anti-inflammatory cytokines, including transforming growth factor-beta [41] and IL-10 [42], modulate central nervous system (CNS) inflammation. The cytokine and chemokine profiles of the rat EAE model are well-documented [23, 34, 35]. There is general agreement that bias toward pro- and/or anti-inflammatory mediators may decide the progression of disease, that is, whether EAE paralysis progresses or not. In certain cases, however, a dual role (protective in lymphoid organs and pathogenic in the CNS) for TNF-alpha has been suggested in mouse models, indicating that TNF-alpha may be either pathogenic or protective in neuroinflammation, depending on its expression by T lymphocytes or myeloid cells, respectively [43]. Even though it is difficult to draw conclusions regarding the roles of particular cytokines in the pathogenesis of EAE, because the network is highly complex, it is generally accepted that pro-inflammatory cytokines are the most important factors for induction of rat EAE.

Factors affecting the remission of rat EAE

Two main factors have been implicated in the process of remission from active EAE in Lewis rats at the cellular level *in vivo*. One is the elimination of inflammatory cells, including T cells, possibly through apoptosis. The other is the activation of regulatory cells, including regulatory T cells [44], macrophages [32, 33], and natural killer cells [45]. These cell types are known to secrete mediators and/or counteract neuroinflammation, contributing to neuroprotection.

Apoptosis of T Cells

Apoptosis has been suggested to be involved in the recovery from rat EAE. This is because many T cells are apoptotic at the peak stage of EAE, and few T cells are found in the spinal cord at the recovery stage of EAE [14, 46, 47]. Furthermore, it has been shown that microglia induce T cell apoptosis in rat EAE [48, 49]. This is a plausible scenario, as inflammation in the CNS will be ameliorated by the loss of causative cells that secrete pro-inflammatory cytokines.

Regulatory T Cells

Regulatory T cells (T-reg) have been implicated in EAE remission [44, 50-52]. Recently, it was reported that increased numbers of Th17 and T-reg lymphocytes were associated with EAE remission in the rat [50, 51]. There is agreement that regulatory T cells secrete anti-inflammatory cytokines, which counteract pro-inflammatory cytokines.

Morphologically, what are presumed to be regulatory T cells have been found in the subarachnoid space (SAS) of rat acute EAE [12, 53, 54]. Two different phenotypes of $CD4^+$ T cells, either $CD45RC^+$ or $CD45RC^-$, have been detected in the SAS at the early stage of EAE [12]. In the SAS, although both cell types infiltrated at the same time in the early stage of EAE, encephalitogenic T cells, which were $CD45RC^-$ (OX22-negative), preferentially infiltrated the parenchyma, followed by $CD45RC^+CD4^+$ T cells at the peak stage. These findings suggest that regulatory T cells infiltrate the EAE lesion, with encephalitogenic T cells in the SAS at the induction stage of rat EAE, and compete with the encephalitogenic T cells, consequently contributing to recovery from EAE via the secretion of anti-inflammatory mediators.

Macrophages

In rat tissues, various types of macrophages have been studied using a series of ED-specific monoclonal antibodies [55, 56]. Two macrophage phenotypes have been identified: pro-inflammatory, classically activated macrophages (M1 phenotype); and immunomodulatory, alternatively activated macrophages (M2 phenotype) [57].

Macrophages are regarded as an important cell type at the induction stage of rat EAE [32, 33], because blocking of macrophages ameliorated the condition. In addition, increased numbers of macrophages have been associated with

http://dx.doi.org/10.5115/acb.2012.45.3.141

the severity of rat acute EAE [22], and macrophages are associated with increased expression of pro-inflammatory mediators, including TNF-alpha and inducible nitric oxide synthase (iNOS) [22]. Macrophages that express TNF-alpha and/or iNOS are classified as classically activated macrophages, or M1 phenotype cells (Fig. 1).

In previous rat EAE studies, macrophages/activated microglia, but not unstimulated microglia in normal rat CNS tissue, were shown to be positive for ED1, while $ED2^{+}$ macrophages were localized mainly in perivascular lesions [55], suggesting that phenotypic differences exist in rat EAE lesions.

Neuropathologically, it is evident that hematogenous macrophages (presumably of the classically activated M1 phenotype) preferentially infiltrate with autoimmune T cells in rat acute EAE [12]. At the EAE induction stage, microglia proliferate in response to T cell infiltration [12, 58]. Even though the number of microglia was increased in rat EAE lesions, the pathological lesions were diminished. This finding suggested that some cellular factors, in addition to regulatory T cells, contribute to EAE modulation. In support of this, activated microglia and/or macrophages [49, 59] were suggested as candidate cell types involved in EAE remission because microglia are activated by expression of CD4-modulated rat EAE [60], and amelioration of clinical EAE was achieved by administration of M2 activated monocytes [61]. Furthermore, even though microglia are known to release potentially cytotoxic molecules, such as pro-inflammatory cytokines, reactive oxygen intermediates, and proteinases [62], their role may be either beneficial or detrimental, depending on the neuropathological conditions [62, 63]. Thus, it is suggested that either activated microglial cells or M2 macrophages, or both, are involved in EAE remission in acute monophasic rat EAE. In a recent study, the presence of M2 macrophages was further evaluated in rat active EAE [11]. In brief, M1 macrophages were predominant at the early stage of EAE, while the proportion of M2 macrophages overwhelmed that of M1 cells at the peak stage and remained high during the recovery stage. Phenotypic differentiation from M1 to M2 macrophages, or activation of microglial cells to the M2 phenotype at the peak stage of EAE remains an area of speculation. The functional role of M2 macrophages was confirmed because type II monocytes have been shown to modulate T cell-mediated mouse EAE through the differentiation of naïve T (Th0) cells into Th2 and $CD4^{+}CD25^{+}FoxP3^{+}$ regulatory T cells [64]. In either case, the increased numbers of M2 macrophages, through the secretion of anti-inflammatory mediators such as activin A [11], may play a role in recovery from rat EAE. To summarize, the data have shown that this bias toward M2 macrophage activation is at least associated with the amelioration of rat active EAE.

Many molecules have been found in $ED1^{+}$ macrophages in rat active EAE, including osteopontin [65], erythropoietin [66], heat-shock protein 27 [67], nitric oxide synthase [46], and arginase-1 [11]. Some, including erythropoietin [68] and osteopontin [69], have been shown to protect neurons in neurodegenerative disease models, even though the role of

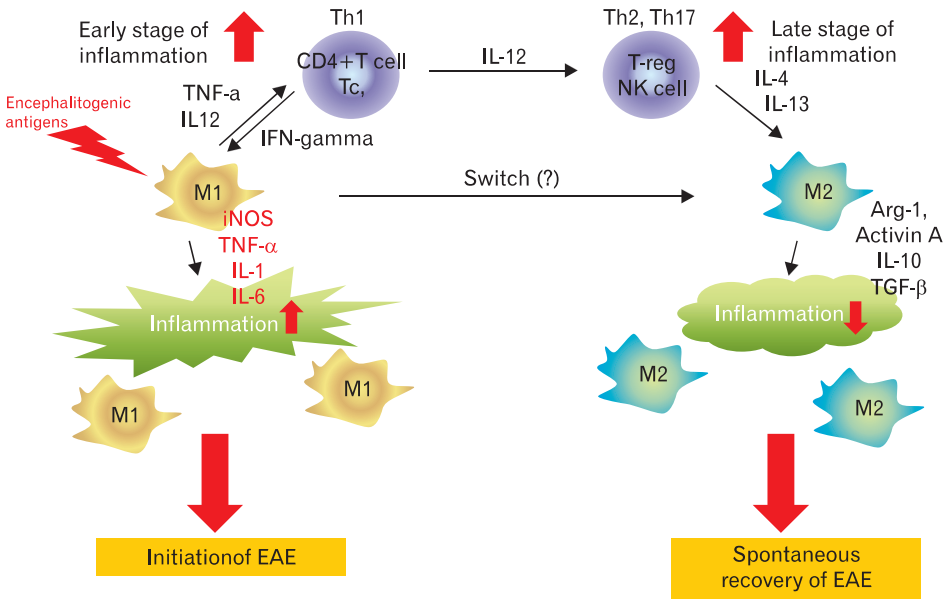

Fig. 1. Schematic diagram of the putative role of each macrophage phenotype in active monophasic experimental autoimmune encephalomyelitis (EAE) in Lewis rats. EAE is mediated by $CD4^{+}$ Th1 cells and is further accelerated by classically activated macrophages (M1), while spontaneous recovery from rat EAE is associated with regulatory T cells and alternatively activated macrophages (M2). IFN, interferon; IL, interleukin; iNOS, inducible nitric oxide synthase; TGF, transforming growth factor; TNF, tumor necrosis factor.

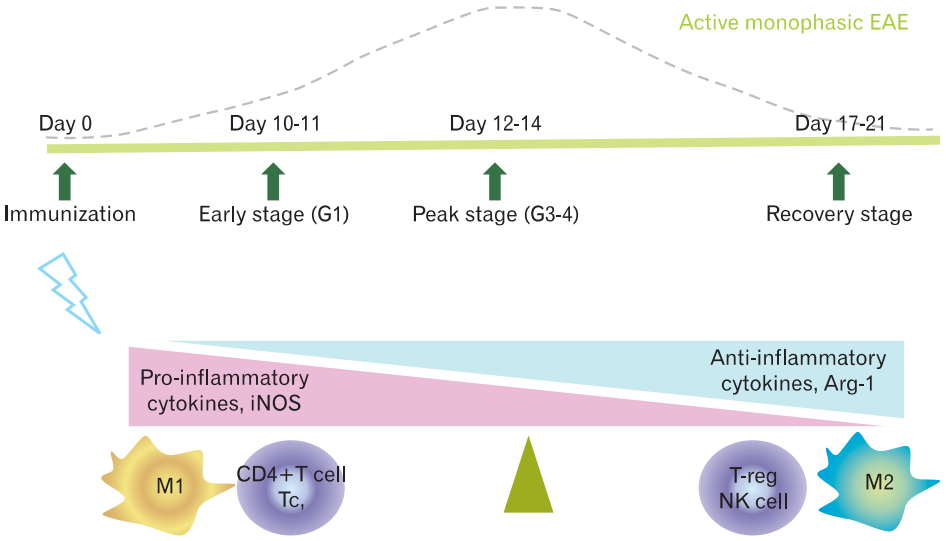

Fig. 2. Schematic diagram of the relationship between clinical signs and inflammatory cells and their products in active monophasic experimental autoimmune encephalomyelitis. Clinical signs largely match the patterns of inflammatory molecules. iNOS, inducible nitric oxide synthase; NK, natural killer; T-reg, regulatory T cell.

osteopontin remains unclear in autoimmune disease models [70]. Thus, a particular macrophage population in rat EAE seems to be a "supplier" of neuroprotective molecules.

The relationship between iNOS and arginase-1, a competitive enzyme for iNOS, will be further discussed (Fig. 2). Upon activation in EAE, macrophages in the lesions are immunopositive for iNOS, which generates nitric oxide through L-arginine. Physiological levels of nitric oxide are beneficial, but can be transiently harmful in rat EAE, when autoimmune T cells infiltrate at the time of EAE induction [71]. However, nitric oxide is also regarded as an EAE-resistant molecule [6, 72], suggesting that the timing of inhibition of iNOS is associated with disease suppression. It is believed that iNOS activity is quickly exhausted in macrophages once activated. Arginase, a competitive enzyme of iNOS, then substitutes for the catalysis of L-arginine, resulting in less production of nitric oxide in the CNS, during recovery-stage EAE [11]. Since some macrophages express both iNOS and arginase, and the expression of iNOS in macrophages is inversely related to that of arginase, it is possible that the phenotypic changes from hematogenous M1 macrophages to M2 macrophages may occur within the same cell in rat EAE. However, it remains possible that phenotypically differentiated M2 macrophages infiltrated alongside hematogenous M1 macrophages. Another possibility is that M2 macrophages originated from proliferation of microglial cells, because microglia were shown to proliferate in rat EAE [14, 58], and alternative activation of microglial cells occurred via IL-4 in a mouse EAE model [73]. A similar result was found in a rat spinal cord injury model in which the majority of macrophages were found to be proliferating [74]. An inverse relationship between iNOS and arginase [75] was identified in a model of spinal cord injury in rats. Taken together, data from the two models of spinal cord inflammation suggest that M2 macrophages may originate from either hematogenous monocytes (via phenotypic differentiation) or activated microglial cells. Both sources of M2 macrophages have been associated with modulation of EAE through the secretion of immunomodulatory molecules, including activin A, in the rat active model. The neuroprotective capacity of macrophages in rat EAE lesions represents a distinct story, but is also beneficial for remission of rat EAE.

Conclusions and Prospective

Active monophasic EAE in Lewis rats has been extensively studied in the past few decades as a model of human autoimmune disease. Many studies have demonstrated the immunological nature of autoimmune T cells and bystander cells during the initial stage of autoimmune inflammation in the target organ, the spinal cord. Since this model lacks demyelination of CNS tissues, the research has focused mainly on T cell immunology. In the field of T cell immunology, rat EAE has served as a good model for the study of pro- and anti-inflammatory cytokines, depending on the disease status, and will in future be utilized for pilot studies of anti-inflammatory drugs. Furthermore, this review has emphasized the involvement of alternatively activated M2 macrophages in remission from EAE and pointed out their importance in recovery from rat EAE, which suggests a

http://dx.doi.org/10.5115/acb.2012.45.3.141

promising strategy for the treatment of autoimmune diseases. Since EAE lesions were protected from further autoimmune attack, and numerous neuroprotective mediators were upregulated at both the peak and recovery stages of EAE, rat EAE is a useful model for the study of both neuroprotection and neuroinflammation.

Acknowledgements

This work was supported by the research grant of the Jeju National University in 2011.

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